Environment-dependent intralocus sexual conflict in a dioecious plant

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Summary

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• Intralocus sexual conflict is a form of conflict that does not involve direct interactions between males and females. It arises when selection on a shared trait with a common genetic basis differs between the sexes. Environmental factors, such as resource availability, may influence the expression and evolutionary outcome of such conflict.

• We quantified the genetic variance-covariance matrix, **G**, for both sexes of *Silene latifolia* for floral and leaf traits, as well as the between-sex matrix, **B**. We also quantified selection on the sexes via survival for 2 yr in four natural populations that varied in water availability.

• Environment-dependent intralocus sexual conflict exists for specific leaf area, a trait that is genetically correlated between the sexes. Males experienced significant negative selection, but only in populations with relatively limited water availability. Females experienced weakly positive or significant stabilizing selection on the same trait. Specific leaf area is genetically correlated with flower size and number, which are sexually dimorphic in this species.

• The extent of intralocus sexual conflict varied with the environment. Resolution of such conflict is likely to be confounded, given that specific leaf area is highly genetically integrated with other traits that are also divergent between the sexes.

Introduction

Sexual conflict has historically been defined as any direct antagonistic interaction between males and females that maximizes the fitness of one sex at the expense of the other (Parker, 1979; Chapman, 2006). Examples within the animal kingdom are numerous, from toxic ejaculates in *Drosophila* to traumatic insemination in spiders (Chapman *et al.*, 1995; Arnqvist & Rowe, 2005; Rezac, 2009). However, another form of sexual conflict – one that manifests itself in the absence of direct interactions between males and females – has recently gained attention (Chapman *et al.*, 2003; van Doorn, 2009). This second form of sexual conflict, termed intralocus sexual conflict, has been experimentally documented for a variety of traits in animals, including weaponry in Soay sheep (*Ovis aries*) (Robinson *et al.*, 2006), adult locomotory activity and dessication resistance in *Drosophila* (Long & Rice, 2007), and immune defense in lizards (Svensson *et al.*, 2009).

Intralocus sexual conflict arises from the inherent problem of exposing a common genetic background to the divergent reproductive strategies of males and females. It occurs when a high between-sex (intersexual) genetic correlation constrains an individual's response to genotype × sex interactions for fitness (Bedhomme & Chippindale, 2007; Bonduriansky & Chenoweth, 2009; Cox & Calsbeek, 2009). Intralocus sexual conflict can occur when one sex, but not the other, experiences significant selection on a shared trait (Day & Bonduriansky, 2004), but will be strongest when males and females experience opposing selective pressures, for example selection gradients with opposite sign (Cox & Calsbeek, 2009). Because this form of conflict is indirect, it may also occur in plants with gender dimorphism (e.g. gynodioecious or dioecious). For example, results consistent with intralocus sexual conflict have been obtained for the sexual morphs of the gynodioecious strawberry (*Fragaria virginiana*): spring leaf number and size were found to be genetically correlated between hermaphrodites and females (0.22 and 0.36, respectively), and fertility selection favored few, large leaves in hermaphrodites and the reverse in females (Ashman, 2005).

The expression and intensity of intralocus sexual conflict may vary with environmental conditions if selection depends on resource availability, population density, or other ecological factors. Consequently, researchers have addressed how environmental variability influences sexual conflict, as changes in environmental conditions have the potential to redraw the adaptive landscape for a shared trait, but such studies are few in number (Fricke *et al.*, 2009).

In this study, we focus on whether intralocus sexual conflict occurs and is dependent on the environment in the dioecious perennial *Silene latifolia*. This species exhibits sexual dimorphism in a variety of morphological, life-history, and ecophysiological traits (Delph, 2007; Delph *et al.*, 2010) and lives in a wide variety of environmental conditions (Taylor & Keller, 2007). We investigated specific leaf area (SLA; leaf area/mass), a measure of leaf thickness that is known to impact water use in many species via its influence on leaf gas-exchange rates (Meziane & Shipley, 2001). We also investigated flower-size traits, which are highly sexually dimorphic in *S. latifolia*. They are also negatively genetically correlated with the number of flowers a plant produces (Steven *et al.*, 2007) and leaf gas-exchange rates (Delph *et al.*, 2005).

Intralocus sexual conflict requires that a trait have a shared genetic basis in the two sexes. To determine this, we first measured leaf and flower traits in a quantitative-genetic crossing experiment in the glasshouse, and estimated the genetic variance-covariance matrices for each sex, $\mathbf{G}_m$ and $\mathbf{G}_f$, and the between-sex matrix, $\mathbf{B}$, using the animal model. This allowed us to quantify heritabilities, as well as among-trait and between-sex genetic correlations. The second component of a study on intralocus sexual conflict requires estimation of selection gradients for the two sexes, and, because we were interested in how the environment might influence these gradients, this required that we estimate selection gradients in multiple populations. Hence, we also performed a phenotypic selection study to determine whether viability selection on any of these traits differed between the sexes in the wild. Neither fecundity nor sexual selection was investigated. These analyses were performed over two successive years in four separate populations that were specifically chosen because they varied in the amount of precipitation during the flowering season.

Our results provide evidence of intralocus sexual conflict for SLA, but only in populations with relatively low rainfall. We discuss these results in terms of the correlation between sexual dimorphism and intralocus sexual conflict, a genetic perspective, and why males have thinner leaves than females when having thin leaves leads to death in males.

Materials and Methods

Silene latifolia Poir. (Caryophyllaceae) is a short-lived, herbaceous perennial that frequently flowers in its first year, producing scented white flowers primarily pollinated by the nocturnal moth *Hadena bicuris* (Jurgens *et al.*, 1996). Sexual dimorphism exists in several morphological traits in this species, the most extreme being flower number, with males making many more flowers than females (Delph, 2007). Moreover, the more a trait is phenotypically correlated with flower number, the more sexually dimorphic it is (Delph *et al.*, 2002). The sexes are also sexually dimorphic for ecophysiological and life-history traits, with males having higher rates of leaf gas exchange, thinner leaves, and shorter lives compared with females (Delph, 2007; Delph *et al.*, 2010).

Estimating $\mathbf{G}$ and $\mathbf{B}$ matrices in the glasshouse

Seeds from each of 103 capsules collected from a wild population in Giles County, Virginia were grown in an Indiana University glasshouse and used as parents in a breeding design in which each mother was mated to three fathers, and vice versa, to give 150 full-sib families. For this study, we used seeds from 120 families. Seeds were planted in a 50 : 50 mix of Metromix (Scotts-Sierra Horticultural Products, Marysville, OH, USA) and sterilized field soil in celled trays in a pollinator-free glasshouse. After germination, eight plants per family were transplanted into 5-inch clay pots filled with the same soil mix. We assigned the plants randomly to rotation groups and moved the groups weekly among benches within the glasshouse to reduce positional effects. Plants were treated with Marathon brand pesticide (OHP Inc., Mainland, PA, USA) before flowering began and were fertilized every 10 d with half-strength 20 : 20 : 20 Peter's fertilizer (Scotts-Sierra Horticultural Products).

All traits were measured on three or more female plants per family, with the exception of four families in which only two female plants flowered and one family in which only one female plant flowered. Three or more males were measured in 53% of families. Twenty-one per cent of families only produced two males, while another 21% only produced one male. Five per cent of families produced no males. The sixth flower produced was collected the morning after it opened, dried to constant weight in a 60°C drying oven, and weighed to an accuracy of 1 mg. Thirty days after a plant opened its first flower, we measured the leaf area of a leaf at the second node down on the stem from the first flower with a portable leaf-area meter (Li3000-A; Li-Cor, Lincoln, Nebraska, USA). In the event that this leaf was damaged, we used the third leaf down. These leaves were then dried to constant weight in a drying oven and weighed. We calculated SLA as leaf area per mass ($cm^2 \, g^{-1}$). We determined the total number of flowers that had been produced 30 d after the first flower opened by counting open flowers, flowers that would open that evening, and pedicels of flowers that had fallen off.

We compared traits between males and females using pooled-samples *t*-tests to determine if the traits were

sexually dimorphic. The sexual dimorphism index (SDI) was measured as: (trait mean of larger sex/trait mean of smaller sex) − 1 (Lovich & Gibbons, 1992). By convention, when the male mean is larger than the female mean for a given trait, the SDI is negative; conversely, when the female trait is larger, the SDI is positive.

We estimated the additive-genetic variance-covariance matrix (**G**) for each sex for flower mass, flower number, and SLA to examine integration among these traits. We ln-transformed flower mass and flower number to make them normally distributed. We treated male and female values for a given trait as separate traits in the analysis. We used the software package VCE (v. 6.0.2; Neumaier & Groeneveld, 1998), which allowed us to use the 'animal' model (in which the estimation is conducted at the individual level) and restricted maximum likelihood (REML). Because REML is not sensitive to unbalanced sample sizes within families or missing data (Lynch & Walsh, 1998), we were able to include families with biased sex ratios in the analysis. We included date of flowering and rotation group in the model to remove the variance caused by these factors before the partitioning of genetic variance. We used VCE to estimate G_m, G_f, **B**, heritabilities, additive-genetic correlations between all traits, and standard errors of these measures. These values were estimated from a total of 618 plants in 120 families. We tested each estimate within the genetic matrix against a null hypothesis of zero using a standard t-test with $n − 2$ degrees of freedom (one-tailed for heritability estimates and two-tailed for genetic correlations). We set n at 262 (the smallest sample size) to determine the degrees of freedom in all significance tests, and applied a sequential Bonferroni correction. We also tested the between-sex (intersexual) genetic correlations for traits against a null hypothesis of 1, again using a one-tailed test and 262 degrees of freedom.

Phenotypic measurements in the wild

Data collection was carried out on naturally growing plants in two populations outside of Alençon, France (hereafter called F2 and F3; F1 was mown and therefore abandoned early in the study) and two populations near Vieira do Minho, Portugal (thereafter called P1 and P2) over the summers of 2005 and 2006. While all four populations experienced, on average, the same temperature during the flowering season, F2 and F3 received over twice as much rainfall as P1 and P2 (Alençon: 160.4 mm in 2005 and 149.8 mm in 2006 vs Vieira do Minho: 76.9 mm in 2005 and 72.4 mm in 2006), which is typical for these areas (http://www.cpc.ncep.noaa.gov/). As a result, individuals in the populations in Portugal are expected to be more water-limited. In each year, *S. latifolia* individuals were identified and tagged before the flowering season, sexed at the onset of flowering, and monitored for survival until the end of the

flowering season. The height of the main flowering stalk (HF) was measured from the ground to the node of the first flower, soon after flowering began. At this time, calyx width (CW), calyx length (CL), and petal-limb (PL) length were also measured using digital calipers on three flowers per plant, and the three values for each trait were averaged for each individual. In addition, SLA was measured as above in the **G**-matrix experiment, 1 month after flowering began. As a consequence, the sample sizes for SLA were sometimes smaller than for the other traits. This reduction occurred for two reasons: either the targeted leaf had shriveled and dried by this time or the plant was dead. Our estimates of selection on SLA are probably conservative, because plant death was more evident for males than females. For example, of the plants whose SLA could not be measured in P2 in 2005, only 2% of the males survived the flowering season, whereas 26% of the females survived. Differences between the sexes were tested with t-tests and the sexual dimorphism index for each trait was calculated as above, separately for each population and year.

Multicollinearity between traits can bias results in logistic regression models used to estimate selection gradients (Mitchell-Olds & Shaw, 1987; Rausher, 1992). As all three floral-size traits (calyx length, calyx width, and petal-limb length) were highly and significantly correlated ($r > 0.5$; $P < 0.05$), a principal component analysis was carried out, and the first principal component, where all the traits loaded positively (Supporting Information Table S3), was taken as a composite measure of flower size and was used in all selection analyses as an independent variable (SIZE).

Viability selection in the wild

Selection analyses using survival as our fitness-related variable were carried out separately for each sex in each population. As values for survival are binary, we used logistic regressions to estimate selection gradients (Schluter, 1988; Janzen & Stern, 1998). All the morphological traits were standardized to have a mean of zero and a standard deviation of 1 (Lande & Arnold, 1983; Janzen & Stern, 1998). The square of these terms as well as the cross-product of SLA and flower size were also calculated. The dependent variable, survival, was not transformed.

Studies of selection on correlated characters obtain two separate but related terms: selection differentials and selection gradients. Selection differentials, which measure both the direct and indirect responses of a trait to selection, were calculated as the covariance between a standardized trait and fitness (Arnold & Wade, 1984). Linear selection gradients, which describe the strength of direct selection on a trait, were calculated using a logistic regression model. While logistic regression models are more appropriate when the fitness measure is binary, the coefficients (α) they generate cannot be compared directly to selection gradients obtained through

multiple least-squares regressions (Schluter, 1988; Brodie *et al.*, 1995). Consequently, we transformed the logistic regression coefficients and obtained $\beta_{avggrad}$, which is comparable to the linear selection gradient (β) obtained through multiple regressions (Janzen & Stern, 1998). Nonlinear selection gradients (γ), which describe nonlinear selection on a trait, were calculated by adding the cross-product and squared standardized traits to the logistic regression. The logistic regression coefficients for significant squared terms were doubled to obtain the nonlinear selection gradients (Lande & Arnold, 1983). Nonlinear selection gradients by themselves cannot be taken as evidence of stabilizing or disruptive selection, as these gradients may be significant even if the selection surface lacks a local maximum or minimum, respectively. Consequently, the fitness surface for a particular trait should be visually represented whenever the regression model yields significant nonlinear gradients. To visually represent the form of selection on a trait, we used univariate cubic spline, nonparametric regressions, which avoid making *a priori* assumptions about the shape of the fitness surface (Schluter, 1988; Brodie *et al.*, 1995). A smoothing parameter (λ) was chosen by minimizing the generalized cross-validation scores.

Some traits were found to be under selection in the populations in Portugal. Hence, an analysis of covariance was used to assess differences between the sexes and the two Portuguese populations in patterns of selection. All statistical analyses were carried out with the R 2.10.0 statistical package (The R Development Core Team, R Foundation for Statistical Computing, available at http://www.R-project.org/index.html).

Joint index for the intensity of sex-specific viability selection

Until recently, most researchers have evaluated the intensity of sexually antagonistic selection as the absolute difference between male and female selection gradients (Arnqvist & Rowe, 2005; Cox & Calsbeek, 2009). This method cannot distinguish between concordant and discordant selection, as

the value is always positive. In a new model, sexually antagonistic selection is positive when selection is concordant between the sexes, negative when selection is discordant between the sexes, and zero when selection is absent in one sex (i.e. sex-limited) (Innocenti & Morrow, 2010). The following formula provides a standardized value by which the intensity and outcome of sex-specific selection can be compared across studies:

$$I = \frac{\beta_M \beta_F}{\sqrt{(\beta_M^2 + \beta_F^2)/2}}.$$

Results

The **G** and **B** matrices in the glasshouse

Traits measured for the estimation of the **G** matrices were all significantly sexually dimorphic (Table S1). Males were shorter, produced smaller flowers, and made more flowers than females. Males also had a larger mean SLA (thinner leaves) than females, although sexual dimorphism in this trait was small in comparison to the floral traits.

In general, genetic correlations among flower number, flower mass, and SLA were strong (Table 1). SLA was significantly positively correlated with flower number in both sexes, and this correlation was especially strong in males, indicating that plants with thicker leaves are genetically constrained to have fewer flowers. The correlation between flower number and size was strong and negative in females, indicating a trade-off. All three between-sex genetic correlations were significantly > 0 and < 1 in this matrix (after Bonferroni correction), and SLA was the trait most strongly correlated between the sexes ($r_{mf} = 0.78$).

Viability selection in the wild

All traits measured in the phenotypic selection study were significantly sexually dimorphic in one or more years and one or more populations (Fig. 1 and Table S2). SLA was only significantly different between the sexes in P1 in 2005,

Table 1 Heritabilities (on the diagonal) and genetic correlations (1 SE) between flower mass, flower number, and specific leaf area (SLA) within (top left and bottom right) and between the sexes (top right) in *Silene latifolia*

Males			Females				
Flower mass	Flower number	SLA	Flower mass	Flower number	SLA		
0.36 (0.82)	−0.37 (0.218)	−0.36 (0.210)	*0.69* (0.084)	−0.52 (0.150)	−0.49 (0.136)	Flower mass	Males
	0.38 (0.108)	**0.82** (0.087)	−0.24 (0.126)	*0.64* (0.096)	0.42 (0.135)	Flower number	
		0.52 (0.123)	−0.23 (0.122)	0.49 (0.122)	*0.78* (0.101)	SLA	
			0.72 (0.080)	−**0.85** (0.071)	−**0.61** (0.094)	Flower mass	Females
				0.44 (0.084)	0.57 (0.138)	Flower number	
					0.48 (0.082)	SLA	

Flower mass and flower number were log$_e$-transformed before analysis. Correlations and heritabilities marked in bold are significantly different from 0 at $P < 0.05$ after a sequential Bonferroni correction. Between-sex correlations in italics are significantly < 1 at $P < 0.015$.
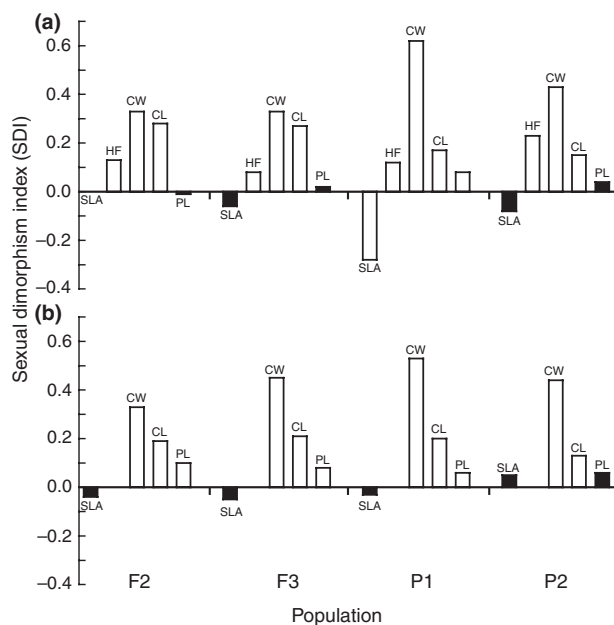
Fig 1 Sexual dimorphism in *Silene latifolia* in the four study populations in 2005 (a) and 2006 (b). Traits include specific leaf area (SLA), height to first flower (HF), calyx width (CW), calyx length (CL), and petal-limb length (PL). A negative sexual dimorphism index (SDI) value indicates that the mean for a trait in males is larger than that of females, and a positive value indicates that the female mean is larger. Closed bars represent a lack of significant sexual dimorphism in a trait, while open bars represent significant sexual dimorphism based on a two-tailed *t*-test (*P* < 0.05).

with males having a larger mean (thinner leaves) than females. Females consistently opened their first flower at a significantly greater height above the ground than males in all populations. Females also consistently had flowers with significantly larger calyces (width and length), but petal-limb length was not consistently significantly different between the sexes.

The proportion of plants that survived during the flowering seasons varied considerably among the populations, being lowest in P1 in 2005 and highest in F3 in 2006 (Table 2). Males tended to die more often than females, and this difference was significant (*P* < 0.05) or nearly so (*P* = 0.06) in four out of the eight comparisons.

The strength and form of selection on the measured traits varied between the sexes and between the two sets of populations with different environmental conditions (F2 and F3 vs P1 and P2; Table 3). Overall, viability selection in F2 and F3 for both years was weak or absent in males and females for all measured traits (Table 3). In fact, logistic regressions using survival as our fitness proxy could not be carried out for these populations in 2006 because most plants survived the flowering season.

By contrast, viability selection in P1 and P2 was strong, sex-specific, and variable across years for SLA and height at first flowering (Table 3). SLA had high and significant neg-

ative selection differentials and selection gradients for males in P1 and P2 for both years. Males with relatively thin leaves were consistently less likely to survive the flowering season (Fig. 2). This selection was seen as strictly directional (e.g. Fig. 2a), with the exception of males in P1 in 2005. Males in this population also had a marginally significant, negative quadratic gradient for SLA. Visualization through a cubic spline revealed that, although the fitness function has a concave curvature, the maximum was highly left skewed, suggesting that selection was predominantly negative and directional (Fig. 2b). Females in Portugal had a significant, negative quadratic gradient for SLA in P1 2006, but visualization for this gradient suggested that the trait was predominately positive and directional, given the absence of a true maximum (Fig. 2c). Females also had a significant positive selection differential and gradient on the height of their first flower in P1 for 2005. Flower size was not under viability selection in either P1 or P2 in either year for either sex. Finally, in several instances selection differentials were significant, but their corresponding selection gradients were not (Table 3).

When comparing selection between the sexes and between P1 and P2 using analysis of covariance, we found a significant interaction between sex and SLA (sex × SLA) in both years (2005: Wald χ^2 = 10.84, df = 1, *P* < 0.001; 2006: Wald χ^2 = 8.89, df = 1, *P* = 0.003), indicating that selection consistently differed between the sexes for this trait in the two populations in Portugal. All other interactions (e.g. sex × flower size, population (P1 vs P2) × SLA, population × flower size, sex × height at flowering, and population × height at flowering) were nonsignificant.

Joint index for sexually antagonistic selection

The two populations in France (F2 and F3) lacked any significant selection gradients, and as a result, had no relevant joint indices. For the populations from Portugal, joint indices were calculated for all five cases in which the directional selection gradient was significant for at least one sex in a given year. Selection differed between the sexes for SLA in both P1 and P2 across years. The selection gradients were significantly negative for males and weakly positive for females in three cases (P1 2005, P1 2006 and P2 2005), leading to joint indices (*I*) of −0.05 to −0.06 (Table 3). In the fourth case (P2 2006), *I* was zero, with selection significantly against males with larger values of SLA, but essentially zero for females. A significant, positive joint index for height of the first flower (HF) was found in P1 in 2005, implying concordant selection (*I* = 0.04).

Discussion

Intralocus sexual conflict can be complicated by variable environmental conditions that redefine the adaptive land-

Table 2 Descriptive information for populations of *Silene latifolia*, with data presented separately for males and females

Site	Year	Sex	Proportion survival	Specific leaf area (cm² g⁻¹)	Height to node of first flower (mm)	Calyx width (mm)	Calyx length (mm)	Petal-limb length (mm)
F2	2005	M	0.47[A]	139.9 ± 4.31 (52)	55.2 ± 0.82 (126)	6.6 ± 0.07 (125)	14.7 ± 0.11 (125)	12.0 ± 0.14 (125)
		F	0.70	140.5 ± 4.60 (78)	62.2 ± 1.01 (129)	8.8 ± 0.12 (96)	18.8 ± 0.20 (96)	11.9 ± 0.16 (96)
	2006	M	0.96	145.7 ± 7.82 (281)		5.7 ± 0.06 (283)	13.8 ± 0.09 (283)	9.6 ± 0.10 (283)
		F	0.94	141.2 ± 5.97 (249)		7.6 ± 0.09 (257)	16.5 ± 0.15 (257)	10.6 ± 0.10 (257)
F3	2005	M	0.53[B]	148.7 ± 9.14 (23)	62.0 ± 1.19 (128)	6.4 ± 0.07 (128)	14.8 ± 0.13 (128)	10.7 ± 0.13 (128)
		F	0.73	140.4 ± 8.52 (34)	67.2 ± 1.32 (122)	8.5 ± 0.13 (61)	18.8 ± 0.25 (61)	10.9 ± 0.22 (61)
	2006	M	0.98	149.3 ± 6.86 (207)		4.7 ± 0.07 (207)	13.7 ± 0.09 (207)	8.9 ± 0.10 (207)
		F	1.00	141.6 ± 4.93 (26)		6.8 ± 0.28 (26)	16.6 ± 0.37 (26)	9.6 ± 0.35 (26)
P1	2005	M	0.13	175.5 ± 6.86 (45)	53.3 ± 1.13 (136)	6.1 ± 0.09 (103)	13.6 ± 0.13 (103)	7.4 ± 0.10 (103)
		F	0.20	135.3 ± 5.23 (33)	59.2 ± 1.14 (145)	9.9 ± 0.16 (71)	16.0 ± 0.16 (71)	7.9 ± 0.14 (70)
	2006	M	0.77	154.4 ± 2.96 (151)		5.9 ± 0.07 (151)	13.6 ± 0.13 (151)	8.1 ± 0.11 (151)
		F	0.82	150.1 ± 3.88 (129)		9.0 ± 0.15 (129)	16.3 ± 0.18 (129)	8.6 ± 0.11 (129)
P2	2005	M	0.37[C]	179.4 ± 8.51 (47)	56.1 ± 1.45 (92)	6.5 ± 0.07 (92)	14.4 ± 0.13 (92)	7.7 ± 0.10 (92)
		F	0.54	166.2 ± 5.01 (60)	70.6 ± 1.83 (92)	9.3 ± 0.12 (81)	16.6 ± 0.18 (81)	7.9 ± 0.11 (81)
	2006	M	0.63[D]	177.7 ± 8.71 (30)		6.4 ± 0.19 (30)	14.0 ± 0.29 (30)	7.8 ± 0.23 (30)
		F	0.84	186.8 ± 9.14 (50)		9.2 ± 0.23 (50)	15.8 ± 0.33 (50)	8.3 ± 0.22 (49)

Data are mean ± 1 SE for males (M) and females (F) from France (F2 and F3) and Portugal (P1 and P2) in 2005 and 2006. Superscripts denote cases in which males were significantly less likely to survive compared with females from the same population/year: [A]χ^2 = 11.21, $P < 0.001$; [B]χ^2 = 7.80, $P < 0.001$; [C]χ^2 = 5.42, $P = 0.02$; [D]χ^2 = 3.35, $P = 0.06$.

Table 3 Summary of selection differentials (S/C), transformed selection gradients (β/γ_{AVG}), and the joint index (*I*, a measure of sexual conflict) for the different sites and years for males and females of *Silene latifolia*

Trait	Males S/C	Males β/γ_{AVG} ± SE	Females S/C	Females β/γ_{AVG} ± SE	*I*	Males S/C	Males β/γ_{AVG} ± SE	Females S/C	Females β/γ_{AVG} ± SE	*I*
	France F2 2005					France F3 2005				
SLA	0.27	0.05 ± 0.04	−0.15	−0.05 ± 0.06	−0.05	−0.20	−0.14 ± 0.11	−0.14	−0.07 ± 0.10	0.08
HF	−0.11	−0.03 ± 0.14	0.14	0	0	0.18	−0.04 ± 0.13	0.04	−0.07 ± 0.10	−0.05
SIZE	−0.20	−0.08 ± 0.06	0.01	0	0	0.10	0.07 ± 0.17	−0.11	0	0
SLA²	0.03	0.07 ± 0.02	−0.07	−0.03 ± 0.07	−	0.14	0.08 ± 0.15	0.05	0	−
HF²	−0.03	−0.11 ± 0.04	0.17	0.32 ± 0.03	−	−0.11	0.24 ± 0.13	**−0.23***	−0.04 ± 0.03	−
SIZE²	0.04	0.17 ± 0.15	−0.06	−0.01 ± 0.05	−	−0.11	0.19 ± 0.13	0.04	0	−
SIZE × SLA	0.07	−0.08 ± 0.06	−0.06	−0.01 ± 0.06	−	−0.14	0.05 ± 0.11	0.14	−	−
	Portugal P1 2005					Portugal P2 2005				
SLA	**−0.58*****	**−0.21 ± 0.05****	−0.01	0.04 ± 0.02	−0.06	**−0.42****	**−0.28 ± 0.11***	0.11	0.04 ± 0.07	−0.06
HF	**0.30*****	0.03 ± 0.04	**0.29*****	**0.20 ± 0.08***	0.04	0.03	0.08 ± 0.12	0.08	−0.01 ± 0.08	−0.01
SIZE	−0.18	−0.03 ± 0.04	0.13	0.09 ± 0.07	−0.04	0	0	0.18	0.06 ± 0.06	0
SLA²	−0.23	−0.37 ± 0.18	0.03	−0.02 ± 0.05	−	−0.27†	0	0	0.02 ± 0.07	−
HF²	**0.18***	0.09 ± 0.06	0.12	0.09 ± 0.11	−	−0.13	0.51 ± 0.27	0.08	0.12 ± 0.08	−
SIZE²	−0.09	−0.08 ± 0.05	0.04	0.03 ± 0.08	−	**0.25***	0	0.11	0	−
SIZE × SLA	−0.09	−0.16 ± 0.10	−0.12	−0.08 ± 0.10	−	0	0.20 ± 0.11	0.20	0.16 ± 0.09	−
	Portugal P1 2006					Portugal P2 2006				
SLA	**−0.21****	**−0.11 ± 0.04***	0.05	0.04 ± 0.03	−0.05	**−0.63****	**−0.11 ± 0.04***	0	0	0
SIZE	0.04	0	0.08	0.03 ± 0.01	0	0.04	0.01 ± 0.03	−0.02	0	0
SLA²	−0.01	0.02 ± 0.03	−0.13	**−0.04 ± 0.01***	−	**0.59****	0.16 ± 0.39	0.01	0	−
SIZE²	−0.07	−0.06 ± 0.02	−0.05	0	−	0.20	0.25 ± 0.33	0.07	0	−
SIZE × SLA	0	0.01 ± 0.03	−0.06	−0.03 ± 0.02	−	−0.24	0.25 ± 0.35	0.23	0	−

Selection differentials and gradients were not estimated in the French populations in 2006 because of uniformly high survival.
SLA, specific leaf area; HF, height to node of first flower; SIZE, flower size; SIZE × SLA, correlational selection; *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.
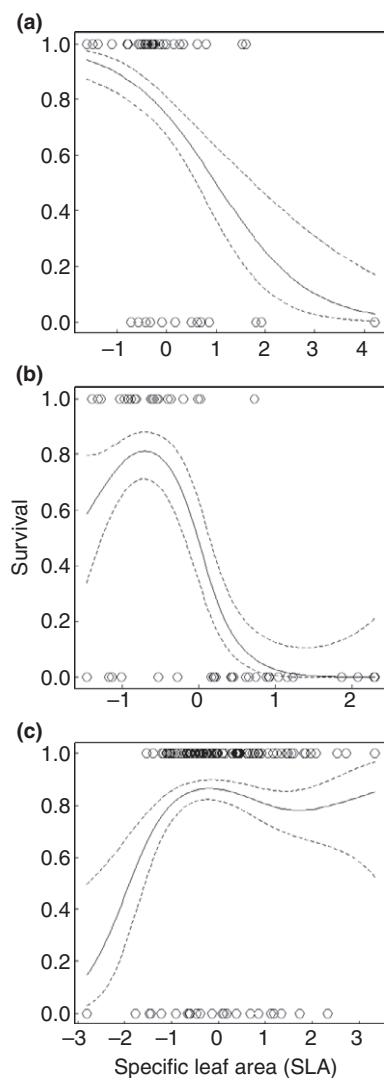
Fig 2 Cubic splines for the relationship between specific leaf area (SLA) and survival (0, died; 1, survived) for *Silene latifolia* (a) males in P2 2005, (b) males in P1 2005, and (c) females in P1 2006. The mean SLA for each population and year combination was set to zero. The solid lines represent the best-fit fitness function, plotted against predicted values with standard errors in the original scale (dashed lines).

also note, however, that viability selection is likely to be a valuable estimate of selection in the wild, as it has been shown to play a major role in wild populations of plants that grow in drought-stressed environments (Latta & McCain, 2009; Carlson *et al.*, 2011).

Viability selection in the wild

Males and females in populations with relatively high rainfall during the flowering season (France: F2 and F3) were not under viability selection for any of the measured traits. In the more arid populations (Portugal: P1 and P2), males that had thinner leaves than the mean tended to die during the flowering season. By contrast, females were either not under viability selection for SLA or under only weak, non-significant positive selection in three out of four of the same arid population/year combinations. In the fourth case, females had a significant negative quadratic selection gradient, suggesting stabilizing selection, but visualization of the gradient showed that this was more positively directional at values less than the mean and relatively flat for values above the mean. These differences in the selection gradients led to indices for discordant selection ranging from 0 to −0.06. The latter value is within the range of indices calculated based on viability selection from over 150 animal studies (Cox & Calsbeek, 2009; Innocenti & Morrow, 2010), which did not differ significantly from the index based on total net selection (Innocenti & Morrow, 2010). In addition to differences in selection gradients for the two sexes for SLA, this trait was highly genetically correlated (0.78) between the sexes in the glasshouse component of the study (and has been found to be controlled by quantitative trait loci (QTL) expressed in both sexes; see the section entitled 'Why a conflict remains: a genetic perspective'). While it is likely that the exact value of the between-sex genetic correlation differs for the four populations studied in the field component, the absolute values need only be significantly greater than zero for intralocus sexual conflict to exist. We conclude that both components needed for intralocus sexual conflict exist for SLA – a significant between-sex genetic correlation and differing sexual gradients for the two sexes, at least in some habitat/year combinations.

Mechanistically, the selection results can be interpreted in light of how SLA is correlated with water loss, together with our knowledge of leaf physiology in *S. latifolia*. In general, thin leaves tend to wilt more and suffer greater water loss than thick leaves (Cunningham *et al.*, 1999; Meziane & Shipley, 2001), and thick leaves have been shown to be adaptive in dry, but not wet habitats, in *Calike edentula* (Dudley, 1996), *Chamaecrista fasciculata* (Etterson, 2004), cork oaks (Ramírez-Valiente *et al.*, 2009; and various white protea species (Carlson *et al.*, 2011). In *S. latifolia*, males have higher leaf gas-exchange rates (Gehring & Monson, 1994; Laporte & Delph, 1996; Delph *et al.*, 2005) and wilt

scape for one or both sexes, making it necessary to include populations that differ environmentally (e.g. Etterson, 2004; Stinchcombe *et al.*, 2010; Carlson *et al.*, 2011). Our results show that environment-dependent intralocus sexual conflict occurs via viability selection on SLA in the wild in *S. latifolia*, a dioecious plant. We note, however, that our study does not take into account how variation in traits likely to affect fitness via mate acquisition and fecundity rather than survival (e.g. flower production) is selected in males and females. A trade-off between viability selection and sexual and/or fecundity selection may occur in one or both sexes, which we discuss in the section entitled 'Why do males (sometimes) have thinner leaves than females?'. We

more when water stressed (L. Delph, pers. obs.) than females. Hence, it is reasonable to hypothesize that males with thin leaves lost more water through their leaves than either males with thicker leaves or females, and this led to death in the water-limited populations.

Height at flowering was sexually dimorphic in all measured populations, with females being taller than males. In addition, relatively tall females in one of the dry populations (P1) in 2005 were better at surviving the flowering season than shorter females. This selection was sexually concordant, rather than antagonistic, as males experienced weak positive selection for height in the same population/year combination. We do not know the heritability of height in this environment, although previous research shows it does have a genetic basis (Delph *et al.*, 2010). However, taller plants may have been better at surviving because height responds plastically to variation in water availability: dry treatments have been shown to result in shorter plants of both sexes (Lyons *et al.*, 1994). Hence, relatively tall plants may be tall because they are more vigorous generally, or else located in microsites with greater water availability.

Flower size, which was included in the analysis as a composite trait, not only was not under direct viability selection in any of the four populations or years (no significant selection gradients), but also was not selected via its correlation with other traits: none of the selection differentials were significant for flower size. We also tested for correlational selection between flower size and SLA (e.g. the possibility for the effect of SLA on survival to depend on flower size) and found no support for such selection. This does not mean that flower size is not under selection in these populations. Sexual and/or fecundity selection could occur for flower size indirectly via its negative genetic correlation with the number of flowers a plant produces. We would have liked to include flower production in our analyses, but doing so was problematic because we did not know the age of the individuals measured, and flower production varies markedly with age (L. Delph and C. Herlihy, unpublished data). In other words, the age of the plant, an unmeasured variable, was a major factor determining flower production. Nevertheless, our results are in accord with those of another study on *S. latifolia* that showed limited directional or stabilizing selection on flower size via fecundity (Wright & Meagher, 2004).

Correlation between sexual dimorphism and intralocus sexual conflict

Previous studies have suggested that the strength of sexual conflict is related to the magnitude of sexual dimorphism of a shared trait, but whether the correlation is positive or negative remains disputed (for a review, see Cox & Calsbeek, 2009). Some studies have demonstrated that sexual dimorphism successfully resolves sexual conflict, so shared traits

that are significantly sexually dimorphic tend to have little or no conflict (Rankin & Arnqvist, 2008). Other studies present a strong positive correlation between sexual conflict and sexual dimorphism, with the strongest conflict present in the most dimorphic traits (Pischedda & Chippindale, 2006). Our results suggest that in *S. latifolia* the presence of sexual dimorphism cannot be taken as evidence for sexually antagonistic selection via viability in the present, nor can the extent of sexual dimorphism be taken as an indicator of the strength of sexual conflict. The height of first flowering was significantly sexually dimorphic across environments and years, and was consistently under concordant positive selection between the sexes. In addition, the least sexually dimorphic trait we measured, SLA, was the only one that experienced significant sexually antagonistic selection. These results suggest that the relationship between sexual dimorphism and intralocus sexual conflict can be complicated, especially when selection is heavily dependent on variable environmental conditions.

Why a conflict remains: a genetic perspective

As several authors have noted, sex-specific expression has been implicated as a possible solution to intralocus sexual conflict (Rhen, 2000; Ellengren & Parsch, 2007; Mank & Ellegren, 2009). Such sex specificity frees the sexes from the genetic constraint imposed by genes that affect the traits in both sexes, allowing the evolution of sexual dimorphism, as well as reducing the conflict between the sexes. With this in mind, Lande (1980) proposed two phases for the evolution of sexual dimorphism: the first occurring relatively rapidly via just such sex-specific gene expression, and the second occurring more slowly as homologous genes (those shared by the two sexes) more recalcitrant to sex-specific modification remained.

A recent genomic architecture study of sexually dimorphic traits of *S. latifolia* mapped one QTL for SLA to the sex-determining region of the Y chromosome, indicating that this gene is differentially expressed depending on which sex it is in (Delph *et al.*, 2010). An additional five QTL were mapped to either the pseudoautosomal regions (PARs) of the sex chromosomes (two QTL) or to the autosomes (three QTL). Of these QTL, three have sex-specific expression (two in one of the PARs and one on an autosome); all three are expressed in males only, and all three make leaves thinner. The allele substitution effect for all three QTL amounts to 84.9, and together they account for 24.8% of the variation in SLA in males. Finally, the remaining two QTL for SLA are expressed equally in both sexes, explaining 6.2% and 4.4% of the variation in SLA, respectively. These results imply that past intralocus sexual conflict involving SLA has been largely resolved via the evolution of sex-specific expression. However, some conflict remains, given the two autosomal QTL with high expression in both sexes

and the high between-sex genetic correlation we found in the present study.

Lastly, a recent review of genes located in the PARs of sex chromosomes lists a small number of studies that have mapped genes for sexually dimorphic traits to the PARs in plants in and animals (Otto *et al.*, 2011). What is unknown is whether any of these traits are sexually antagonistic. This is of interest because it has been argued that genes that have evolved sex-limited expression might remain in the PAR, and thereby affect the evolutionary dynamics of the sex chromosomes, whereas those that have not (and hence remain antagonistic) should move into the nonrecombining region of the sex chromosomes (Scotti & Delph, 2006; Otto *et al.*, 2011). The results in this paper showing intralocus sexual antagonism for SLA, together with the results showing that two QTL for this trait are located in a PAR and are sex-specific in their expression, are the first to support this premise.

Why do males (sometimes) have thinner leaves than females?

As seen in our results, SLA is sometimes, but not always, sexually dimorphic (see also Delph *et al.*, 2002, 2005, 2010). Even when it is significantly dimorphic, the degree of sexual dimorphism is low relative to many other traits (Delph, 2007). Moreover, it is heritable (Table 1), variable among populations (Delph *et al.*, 2002), plastic in both sexes (being reduced in dry treatments relative to wet treatments; Delph & Bell, 2008) and, as seen here, is under viability selection in males inhabiting dry populations. Given that it is males that are under selection to have thicker leaves, it may seem paradoxical that, when the sexes do exhibit significant sexual dimorphism for this trait, it is the males that have thinner leaves. Interpretation of this pattern would be helped by knowledge of how SLA is genetically correlated with other traits, especially those that might impact the fitness of a male through either fecundity or sexual selection, or both. This is especially true for males, as they are more genetically integrated than females (Steven *et al.*, 2007; Delph *et al.*, 2010). In other words, different forms of selection on a trait that is genetically correlated could lead to indirect (correlated) selection on SLA. Importantly, SLA is, as shown here, positively genetically correlated with the number of flowers a plant produces. This matches results from an artificial selection experiment, in which males selected to have larger flowers (more like females) both made fewer flowers and had thicker leaves than control lines (Delph *et al.*, 2004, 2005). Hence, although having thin leaves as a male can lead to a higher probability of death in relatively water-limited environments, it can also lead to the production of more flowers in males that survive. If making a relatively large number of flowers leads to greater siring success (and hence fitness) via enhanced pollinator attraction or the production of more

pollen, this would lead to selection for thinner leaves. We therefore expect the extent of sexual dimorphism in SLA to be a complex relationship between the three forms of selection: viability, fecundity, and sexual selection. Moreover, the relative strength of these forms of selection is likely to vary with environmental heterogeneity. Even if fecundity and sexual selection were to be relatively unvarying, we have shown here that viability selection varies with the environment. Our results and interpretation are in concordance with SLA having significant among-population variability.

Overall, our results suggest that the interaction between traits experiencing sexual conflict and ecological factors can be complicated when these traits have high genetic correlations with other divergent traits that experience their own-sex specific, environment-dependent selection (see also Ashman, 2005). When ecological factors are highly variable, even sex-specific expression might be unable to resolve sexual antagonism.

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References

Arnold SJ, Wade MJ. 1984. On the measurement of natural and sexual selection. *Evolution* **38**: 709–719.

Arnqvist G, Rowe L. 2005. *Sexual conflict*. Princeton, NJ, USA: Princeton University Press.

Ashman T-L. 2005. The limits on sexual dimorphism in vegetative traits in a gynodioecious plant. *American Naturalist* **166**: S5–S16.

Bedhomme S, Chippindale AK. 2007. Irreconcilable differences: when sexual dimorphism fails to resolve sexual conflict. In: Fairbairn DJ, Blanckenhorn WU, Székely T, eds. *Sex, size, and gender roles. Evolutionary studies of sexual size dimorphism*. New York, NY, USA: Oxford University Press, 185–194.

Bonduriansky R, Chenoweth SF. 2009. Intralocus sexual conflict. *Trends in Ecology & Evolution* **24**: 280–288.

Brodie ED III, Moore AJ, Janzen FJ. 1995. Visualizing and quantifying natural selection. *Trends in Ecology & Evolution* **10**: 313–318.

Carlson JE, Holsinger KE, Prunier R. 2011. Plant responses to climate in the Cape Floristic Region of South Africa: evidence for adaptive differentiation in the Proteaceae. *Evolution* **65**: 108–124.

Chapman T. 2006. Evolutionary conflicts of interest between males and females. *Current Biology* **16**: R744–R754.

Chapman T, Arnqvist G, Bangham J, Rowe L. 2003. Sexual conflict. *Trends in Ecology & Evolution* **18**: 41–47.

Chapman T, Liddle LF, Kalb JM, Wolfner MF, Partridge L. 1995. Cost of mating in *Drosophila melanogaster* females is mediated by male accessory protein glands. *Nature* **373**: 241–244.

Cox RM, Calsbeek R. 2009. Sexually antagonistic selection, sexual dimorphism, and the resolution of intralocus sexual conflict. *American Naturalist* 173: 176–187.

Cunningham SA, Summerhayes B, Westoby M. 1999. Evolutionary divergeneces in leaf structure and chemistry, comparing rainfall and soil nutrient gradients. *Ecology* 69: 569–588.

Day T, Bonduriansky R. 2004. Intralocus sexual conflict can drive the evolution of genomic imprinting. *Genetics* 167: 1537–1546.

Delph LF. 2007. The genetic integration of sexually dimorphic traits in the dioecious plant, *Silene latifolia*. In: Fairbairn DJ, Blanckenhorn WU, Székely T, eds. *Sex, size, and gender roles. Evolutionary studies of sexual size dimorphism*. New York, NY, USA: Oxford University Press, 115–123.

Delph LF, Arntz AM, Scotti-Santiange C, Scotti I. 2010. Quantitative trait loci and genomic architecture of sexual dimorphism in the dioecious plant *Silene latifolia*. *Evolution* 64: 2873–2876.

Delph LF, Bell DL. 2008. A test of the differential-plasticity hypothesis for variation in the degree of sexual dimorphism in *Silene latifolia*. *Evolutionary Ecology Research* 10: 61–75.

Delph LF, Gehring JL, Arntz AM, Levri M, Frey FM. 2005. Genetic correlations with floral display lead to sexual dimorphism in the cost of reproduction. *American Naturalist* 166: S31–S41.

Delph LF, Gehring JL, Frey FM, Artnz AM, Levri M. 2004. Genetic constraints on floral evolution in a sexually dimorphic plant revealed by artificial selection. *Evolution* 58: 1936–1946.

Delph LF, Knapczyk FN, Taylor DR. 2002. Among-population variation and correlations in sexually dimorphic traits of *Silene latifolia*. *Journal of Evolutionary Biology* 15: 1011–1020.

van Doorn GS. 2009. Intralocus sexual conflict. *The Year in Evolutionary Biology, Annals of the New York Academy of Sciences* 1168: 52–71.

Dudley SA. 1996. Differing selection on plant physiological traits in response to environmental water availability: a test of adaptive hypotheses. *Evolution* 50: 92–102.

Ellengren H, Parsch J. 2007. The evolution of sex-biased genes and sex-biased gene expression. *Nature Reviews Genetics* 8: 689–698.

Etterson JR. 2004. Evolutionary potential of *Chamaecrista fasciculata* in relation to climate change. I. Clinal patterns of selection along an environmental gradient in the great plains. *Evolution* 58: 1446–1458.

Fricke C, Perry J, Chapman T, Rowe L. 2009. The conditional economics of sexual conflict. *Biology Letters* 5: 671–674.

Gehring JL, Monson RK. 1994. Sexual differences in gas exchange and response to environmental stress in dioecious *Silene latifolia* (Caryophyllaceae). *American Journal of Botany* 81: 166–174.

Innocenti P, Morrow EH. 2010. A joint index for the intensity of sex-specific selection. *Evolution* 64: 2775–2778.

Janzen FJ, Stern HS. 1998. Logistic regression for empirical studies of multivariate selection. *Evolution* 52: 1564–1571.

Jurgens A, Witt T, Gottsberger G. 1996. Reproduction and pollination in central European populations of *Silene* and *Saponaria* species. *Botanica Acta* 109: 316–324.

Lande R. 1980. Sexual dimorphism, sexual selection, and adaptation in polygenic characters. *Evolution* 34: 292–305.

Lande R, Arnold S. 1983. The measurement of selection on correlated characters. *Evolution* 37: 1210–1226.

Laporte MM, Delph LF. 1996. Sex-specific physiology and source-sink relations in the dioecious plant *Silene latifolia*. *Oecologia* 106: 63–72.

Latta RG, McCain C. 2009. Path analysis of natural selection via survival and fecundity across contrasting environments in *Avena barbata*. *Journal of Evolutionary Biology* 22: 2458–2469.

Long TA, Rice WR. 2007. Adult locomotory activity mediates intralocus sexual conflict in a laboratory-adapted population of *Drosophila melanogaster*. *Proceedings of the Royal Society of London Series B* 274: 3105–3112.

Lovich JE, Gibbons JW. 1992. A review of techniques for quantifying sexual size dimorphism. *Growth, Development, and Aging* 56: 269–281.

Lynch M, Walsh B. 1998. *Genetics and analysis of quantitative traits*. Sunderland, MA, USA: Sinauer.

Lyons EE, Miller D, Meagher TR. 1994. Sibship differences in sex ratio and gender dimorphism in *Silene latifolia*. I. Environmental effects. *Journal of Heredity* 85: 196–203.

Mank JE, Ellegren H. 2009. Sex-Linkage of sexually antagonistic genes is predicted by female, but not male, effects in birds. *Evolution* 63: 1464–1472.

Meziane D, Shipley B. 2001. Direct and indirect relationships between specific leaf area, leaf nitrogen and leaf gas exchange. Effects of irradiance and nutrient supply. *Annals of Botany* 88: 915–927.

Mitchell-Olds T, Shaw RG. 1987. Regression analysis of natural selection: statistical inference and biological interpretation. *Evolution* 41: 1149–1161.

Neumaier A, Groeneveld E. 1998. Restricted maximum likelihood estimates of covariances in sparse linear models. *Genetics Selection Evolution* 30: 3–26.

Otto SP, Pannell J, Peichel CL, Ashman T-L, Charlesworth D, Chippindale AK, Delph LF, Guerrero RF, Scarpino SV, McAllister BF. 2011. About PAR: the distinct evolutionary dynamics of the Pseudoautosomal Region. *Trends in Genetics*, in press.

Parker GA. 1979. Sexual selection and sexual conflict. In: Blum NS, Blum NA, eds. *Sexual selection and reproductive competition in insects*. London, UK: Academic Press, 123–166.

Pischedda A, Chippindale AK. 2006. Intralocus sexual conflict diminishes the benefits of sexual selection. *PLoS Biology* 4: e356.

Ramírez-Valiente JA, Lorenzo Z, Soto A, Valladares F, Gil L, Aranda I. 2009. Elucidating the role of genetic drift and natural selection in cork oak differentiation regarding drought tolerance. *Molecular Ecology* 18: 3803–3815.

Rankin DJ, Arnqvist G. 2008. Sexual dimorphism is associated with population fitness in the seed beetle *Callosobruchus maculatus*. *Evolution* 62: 622–630.

Rausher MD. 1992. The measurement of selection on quantitative traits: biases due to environmental covariances between traits and fitness. *Evolution* 46: 616–626.

Rezac M. 2009. The spider *Harpactea sadistica*: co-evolution of traumatic insemination and complex female genital morphology in spiders. *Proceedings of the Royal Society of London Series B* 276: 2697–2701.

Rhen T. 2000. Sex-limited mutations and the evolution of sexual dimorphism. *Evolution* 54: 37–43.

Robinson MR, Pilkington JG, Clutton-Brock TH, Pemberton JM, Kruuk LEB. 2006. Live fast, die young: trade-offs between fitness components and sexually antagonistic selection on weaponry in Soay sheep. *Evolution* 60: 2168–2181.

Schluter D. 1988. Estimating the form of natural selection on a quantitative trait. *Evolution* 42: 849–861.

Scotti I, Delph LF. 2006. Selective trade-offs and sex-chromosome evolution in *Silene latifolia*. *Evolution* 60: 1793–1800.

Steven J, Delph LF, Brodie ED III. 2007. Sexual dimorphism in the quantitative-genetic architecture of floral, leaf, and allocation traits in *Silene latifolia*. *Evolution* 61: 42–57.

Stinchcombe JR, Izem R, Heschel MS, McGoey BV, Schmitt J. 2010. Across-environment genetic correlations and the frequency of selective environments shape the evolutionary dynamics of growth rate in *Impatiens capensis*. *Evolution* 64: 2887–2903.

Svensson EI, McAdam AG, Sinervo B. 2009. Intralocus sexual conflict over immune defense, gender load, and sex-specific signaling in a natural lizard population. *Evolution* 63: 3124–3135.

Taylor DR, Keller SR. 2007. Historical range expansion determines the phylogenetic diversity introduced during contemporary species invasion. *Evolution* 61: 334–345.

Wright JW, Meagher TR. 2004. Selection on flower characters in natural Spanish populations of *Silene latifolia*. *Journal of Evolutionary Biology* 17: 382–395.

Supporting Information

Additional supporting information may be found in the online version of this article.

Table S1 Sample sizes, means, *t*-statistics, and *P*-values of *t*-tests comparing the sexes and the sexual dimorphism index (SDI) for *Silene latifolia*

Table S2 Sexual dimorphism indexes (SDIs) and comparison of the sexes of *Silene latifolia*

Table S3 Loading for the principal components analysis for flower size for each population/year combination

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